

AMENDMENTS

The Examiner is respectfully requested to make the following amendments.

IN THE CLAIMS:

1. (Original) A method for detecting protein-protein interactions in a host cell cytoplasm, the method comprising:
 - a. introducing a first recombinant expression construct encoding a first protein or protein-binding fragment thereof fused with the amino- or carboxyl- terminus of a transcriptional inhibitor;
 - b. introducing a second recombinant expression construct encoding a second protein or protein-binding fragment thereof fused to a cytoplasm localization sequence, wherein upon interaction of the first and second proteins in the cell cytoplasm, said transcriptional inhibitor is localized to the cytoplasm, wherein transcription of a gene operably linked to a promoter that is sensitive to or regulated by said transcriptional inhibitor is increased; and
 - c. detecting said increased transcription of said gene, wherein said protein-protein interaction is detected thereby.
2. (Original) The method of claim 1, wherein the cytoplasm localization sequence is a membrane targeting sequence.
3. (Original) The method of claim 2, wherein the membrane targeting sequences are a myristoylation sequence, mitochondrial outer membrane targeting sequence, or a membrane anchoring sequence.
4. (Original) The method of Claim 3 wherein the myristoylation sequence is MGCTVSTQTIGDESDP (SEQ ID NO:1).
5. (Original) The method of Claim 3 wherein the mitochondrial outer membrane targeting sequence is the N-terminal sequence of Tom70/Mas70 protein, MKSFITRNKTAILATVAATGTAIGAYYYY (SEQ ID NO:3).

6. (Original) The method of claim 1, wherein the second protein is a protein encoded by a cDNA or a member of a cDNA library, wherein said library comprises a plurality of fusion proteins in which the transcription inhibitor protein is fused to each of a plurality of members of said cDNA library in each species of fusion protein comprising said plurality.
7. (Original) The method of claim 1, wherein the second protein is a transcriptional activator protein or one of a multiplicity of proteins that participate in protein-protein interactions to bring about transcriptional activation.
8. (Original) The method of claim 1, wherein the first protein is a protein encoded by a cDNA or a member of a cDNA library, wherein said library comprises a plurality of fusion proteins in which the transcription inhibitor protein is fused to each of a plurality of members of said cDNA library in each species of fusion protein comprising said plurality.
9. (Original) The method of claim 1, wherein the second protein is a transcriptional activator protein or one of a multiplicity of proteins that participate in protein-protein interactions to bring about transcriptional activation.
10. (Original) The method of claim 1, wherein said first or second proteins are detectable or produce detectable metabolites.
11. (Original) The method of claim 1, wherein the gene expressed from the promoter that is sensitive to or regulated by the transcriptional inhibitor is one of a multiplicity of genes that encode detectable proteins.
12. (Original) The method of claim 1, wherein the gene operably linked to a promoter that is sensitive to or regulated by said transcriptional inhibitor is a gene expressed from a GAL4 protein activatable promoter.

13. (Original) The method of claim 12, wherein the gene expressed from the GAL4 protein activatable promoter is one of a multiplicity of genes that encode detectable proteins.
14. (Original) The method of claim 12, wherein the GAL4 protein activatable promoter is one of a multiplicity of promoters that contain a UAS_{GAL} site.
15. (Original) The method of claim 12, wherein the gene operably linked to a promoter that is sensitive to or regulated by said transcriptional inhibitor is one of a multiplicity of genes encoding a detectable product.
16. (Original) The method of claim 12, wherein the transcription inhibitor is Gal80p.
17. (Original) The method of claim 1, wherein the gene operably linked to a promoter that is sensitive to or regulated by said transcriptional inhibitor is a selectable gene, wherein increased expression of said gene confers a growth advantage on the cell or distinguishes the cell in some detectable manner.
18. (Original) The method of claim 16, further comprising:
- d. subjecting the host cell to selective growth conditions; and
 - e. detecting increased growth or survival of said cells under selective growth conditions;
- wherein said protein-protein interaction is detected thereby.
19. (Original) A method for isolating said first or second fusion proteins, the method comprising:
- a. detecting increased expression of the gene operably linked to a promoter that is sensitive to or regulated by said transcriptional inhibitor; and
 - b. isolating said first or second fusion proteins.

20. (Original) The method of claim 19, wherein said first or second proteins comprise one or a plurality of members of a cDNA library, wherein said library comprises a plurality of fusion proteins in which the transcription inhibitor protein is fused to each of a plurality of members of said cDNA library in each species of fusion protein comprising said plurality.

21. (Original) A method for detecting protein-protein interactions in cytoplasm of a cell of a multicellular organism, the method comprising:

- a. introducing a first recombinant expression construct encoding a first protein or protein-binding fragment thereof fused with the amino- or carboxyl- terminus of Gal80p;
- b. introducing a second recombinant expression construct encoding a second protein or protein-binding fragment thereof fused to a cytoplasm localization sequence, wherein upon interaction of the first and second proteins in the cell cytoplasm, said Gal80p is localized to the cytoplasm, wherein transcription of a gene operably linked to a promoter that is sensitive to or regulated by said Gal80p is increased; and

- c. detecting said increased transcription of said gene,

wherein said protein-protein interaction is detected thereby.

22. (Withdrawn) A method for detecting in a cytoplasm of a cell from a multicellular organism a nuclear export sequence (NES), the method comprising:

- a. introducing into the cell a recombinant expression construct encoding a NES-containing protein or NES-containing fragment thereof fused with the amino- or carboxyl-terminus of a transcription inhibitor;
 - b. assaying the cell for expression of a gene operably linked to a promoter that is sensitive to or regulated by said transcriptional inhibitor;
 - c. detecting increased expression of said gene,
- wherein a NES is detected thereby.

23. (Withdrawn) The method of claim 22, wherein the recombinant expression construct encodes a cDNA or a member of a cDNA library, wherein said library comprises a plurality of fusion proteins in which the transcription inhibitor protein is fused to each of a plurality of

members of said cDNA library in each species of fusion protein comprising said plurality, wherein certain of the members of the cDNA species comprise an NES .

24. (Withdrawn) The method of claim 22, wherein the gene expressed from the promoter that is sensitive to or regulated by the transcriptional inhibitor is one of a multiplicity of genes that encode detectable proteins.

25. (Withdrawn) The method of claim 22, wherein the gene operably linked to a promoter that is sensitive to or regulated by said transcriptional inhibitor is a selectable gene, wherein increased expression of said gene confers a growth advantage on the cell.

26. (Withdrawn) The method of claim 23, further comprising:

- d. subjecting the host cell to selective growth conditions; and
- e. detecting increased growth or survival of said cells under selective growth conditions;

wherein said NES is detected thereby.

27. (Withdrawn) A method for isolating a nuclear export sequence (NES), the method comprising:

- a. detecting increased expression of the gene operably linked to a promoter that is sensitive to or regulated by a transcriptional inhibitor in a cell comprising a recombinant expression construct encoding a NES-containing protein or NES-containing fragment thereof fused with the amino- or carboxyl-terminus of a transcription inhibitor; and
- b. isolating said recombinant expression construct comprising said NES from the cell.

28. (Withdrawn) The method of claim 27, wherein said NES comprises one or a plurality of members of a cDNA library, wherein said library comprises a plurality of fusion proteins in which the transcription inhibitor protein is fused to each of a plurality of members of said cDNA

library in each species of fusion protein comprising said plurality, wherein certain of the members of the cDNA species comprise an NES.

29. (Withdrawn) The method of claim 22, wherein the gene operably linked to a promoter that is sensitive to or regulated by said transcriptional inhibitor is a gene expressed from a GAL4 protein activatable gene promoter.

30. (Withdrawn) The method of claim 29, wherein the gene expressed from the GAL4 protein activatable gene promoter is one of a multiplicity of genes that encode detectable proteins.

31. (Withdrawn) The method of claim 29, wherein the GAL4 activatable promoter is one of a multiplicity of promoters that contain a UASGAL site.

32. (Withdrawn) The method of claim 29, wherein the gene operably linked to a promoter that is sensitive to or regulated by said transcriptional inhibitor is one of a multiplicity of genes encoding a detectable product.

33. (Withdrawn) The method of claim 29, wherein the transcription inhibitor is Gal80p.

34. (Withdrawn) The method of claim 29, wherein the gene operably linked to a promoter that is sensitive to or regulated by said transcriptional inhibitor is a selectable gene, wherein increased expression of said gene confers a growth advantage on the cell or distinguishes the cell in some detectable manner.

35. (Withdrawn) The method of claim 34, further comprising:

- d. subjecting the host cell to selective growth conditions, and
- e. detecting increased growth or survival of said cells under selective growth

conditions,

wherein said NES is detected thereby.

36. (Withdrawn) A method for detecting nuclear export sequences (NES) in a host cell cytoplasm comprising:
- a. introducing a gene expressing a protein or fragment thereof fused with the amino- or carboxyl-terminus of Gal80p;
 - b. assaying the cell for expression of a gene operably linked to a promoter that is sensitive to or regulated by Gal80p;
 - c. detecting increased expression of said gene.

wherein an NES is detected thereby.

37. (Withdrawn) The method of claim 36, wherein the recombinant expression construct encodes a cDNA or a member of a cDNA library, wherein said library comprises a plurality of fusion proteins in which the Gal80p is fused to each of a plurality of members of said cDNA library in each species of fusion protein comprising said plurality, wherein certain of the members of the cDNA species comprise an NES.

38. (Withdrawn) A method for isolating a nuclear export sequence (NES), the method comprising:

- a. detecting increased expression of the gene operably linked to a promoter that is sensitive to or regulated by a transcriptional inhibitor in a cell comprising a recombinant expression construct encoding a NES-containing protein or NES-containing fragment thereof fused with the amino- or carboxyl-terminus of Gal80p; and
- b. isolating said NES from the protein fused to Gal80p.

39. (Withdrawn) The method of claim 38, wherein the gene expressed from the promoter that is sensitive to or regulated by the transcriptional inhibitor is one of a multiplicity of genes that encode detectable proteins.

40. (Withdrawn) The method of claim 38, wherein the gene operably linked to a promoter that is sensitive to or regulated by said transcriptional inhibitor is a selectable gene, wherein increased expression of said gene confers a growth advantage on the cell.

41. (Withdrawn) The method of claim 40, further comprising:
- d. subjecting the host cell to selective growth conditions; and
 - e. detecting increased growth or survival of said cells under selective growth conditions;
- wherein said NES is detected thereby.
42. (Withdrawn) A method for detecting a nuclear localization sequence (NLS) in a host cell comprising:
- a. introducing into the cell a recombinant expression construct encoding a NLS-containing protein or NLS-containing fragment thereof fused with the amino- or carboxyl-terminus of a transcription inhibitor;
 - b. assaying the cell for expression of a gene operably linked to a promoter that is sensitive to or regulated by said transcriptional inhibitor;
 - c. detecting decreased expression of said gene,
- wherein an NLS is detected thereby.
43. (Withdrawn) The method of claim 42. wherein the gene operably linked to a promoter that is sensitive to or regulated by said transcriptional inhibitor encodes a product that converts a non-toxic compound to a cytotoxic or cytostatic compound.
44. (Withdrawn) The method of claim 42. wherein the recombinant expression construct encodes a cDNA or a member of a cDNA library, wherein said library comprises a plurality of fusion proteins in which the transcription inhibitor protein is fused to each of a plurality of members of said cDNA library in each species of fusion protein comprising said plurality, wherein certain of the members of the cDNA species comprise an NLS.
45. (Withdrawn) The method of claim 42, wherein expression of the gene operably linked to a promoter that is sensitive to or regulated by said transcriptional inhibitor is inducible when the cell is contacted with an inducing agent.

46. (Withdrawn) The method of claim 45, wherein the transcriptional inhibitor is Gal80p and the inducing agent is galactose.
47. (Withdrawn) The method of claim 42, wherein the gene operably linked to a promoter that is sensitive to or regulated by said transcriptional inhibitor encodes a product that converts a non-toxic compound to a cytotoxic or cytostatic compound and wherein expression of the gene operably linked to a promoter that is sensitive to or regulated by said transcriptional inhibitor is inducible when the cell is contacted with an inducing agent, wherein decreased expression of said gene is detected by cell growth or survival when the cell is contacted with both the inducing agent and the cytotoxic or cytostatic compound.
48. (Withdrawn) A method for detecting a nuclear localization sequence (NLS) in a host cell comprising:
- a. introducing into the cell a recombinant expression construct encoding a NLS-containing protein or NLS-containing fragment thereof fused with the amino- or carboxyl-terminus of Gal80p;
 - b. assaying the cell for expression of a gene operably linked to a promoter that is sensitive to or regulated by Gal80p;
 - c. detecting decreased expression of said gene,
- wherein an NLS is detected thereby.
49. (Withdrawn) A method for isolating a nuclear localization sequence (NLS), the method comprising:
- a. detecting decreased expression of the gene operably linked to a promoter that is sensitive to or regulated by said transcriptional inhibitor and encoded by a recombinant expression construct encoding a NLS-containing protein or NLS-containing fragment thereof fused with the amino- or carboxyl-terminus of a transcription inhibitor; and
 - b. isolating said recombinant expression construct comprising said NLS from the cell.

50. (Withdrawn) A galactose-independent method of activating GAL4 protein regulatable gene expression in multicellular organisms or cells therefrom comprising:
- a. introducing a gene expressing a first protein or fragment thereof fused with the amino- or carboxyl-terminus of Gal80p,
 - b. introducing a gene expressing a second protein or fragment thereof fused to a membrane targeting sequence, and
 - c. treating the multicellular organism or cell therefrom with an induction agent, wherein treatment mediates the cytoplasmic interaction of the first and second proteins, relieving Gal80p inhibition and permitting Gal4p activation of GAL4 protein regulatable, UASGAL - containing promoters,
- wherein the promoters are fused to a heterologous gene.
51. (Withdrawn) The method of claim 50, wherein Gal4p or a fragment thereof is expressed from a chromosomal Gal4p gene or a heterologous Gal4p gene introduced into the multicellular organisms or cells therefrom.
52. (Withdrawn) The method of claim 50, wherein the membrane targeting sequences are a myristoylation sequence, mitochondrial outer membrane targeting sequence, or other membrane targeting/anchoring sequences.
53. (Withdrawn) The method of Claim 52 wherein the myristoylation sequence is MGCTVSTQTIGDESDP (SEQ ID NO:1).
54. (Withdrawn) The method of Claim 52 wherein the mitochondrial outer membrane targeting sequence is the N-terminal sequence of Tom70/Mas70 protein, MKSFITRNKTAILATVAATGTAIGAYYYY (SEQ ID NO:3).
55. (Withdrawn) The method of claim 50, wherein the first protein is any protein encoded by a cDNA.

56. (Withdrawn) The method of claim 55, wherein the first protein is Fpri.
57. (Withdrawn) The method of claim 50, wherein the second protein is any protein encoded by a cDNA.
58. (Withdrawn) The method of claim 57, wherein the second protein is Cnal.
59. (Withdrawn) The method of claim 50, wherein the induction agent is any molecule that mediates the interaction of said first and second proteins.
60. (Withdrawn) The method of claim 50, wherein the induction agent is FK.506.
61. (Withdrawn) The method of claim 50, wherein the GAL4 protein regulatable genes are expressed from UAS_{GAL} containing promoters.
62. (Withdrawn) The method of claim 50, wherein the GAL4 protein regulatable gene is transcribed through any promoter under the control of UAS_{GAL} sequences and the UASGAL-specific transcriptional activator sequences.
63. (Withdrawn) A method of regulatable gene expression in multicellular organisms or cells therefrom comprising:
- introducing a recombinant expression construct encoding Gal80p into the multicellular organism or cell therefrom,
 - introducing a recombinant expression construct encoding Gal3p into the multicellular organism or cell therefrom,
 - introducing a recombinant expression construct encoding Gal4p into the multicellular organism or cell therefrom,
 - introducing a coding sequence operably linked to a promoter sensitive to or regulated by the proteins encoded by the expression constructs of steps a-c,
 - treating the multicellular organism or cell therefrom with an inducing agent,

wherein treatment mediates the cytoplasmic interaction of the Gal3p and Gal80p, relieving Gal80p inhibition and permitting Gal4p activation of the coding sequence operably linked to the promoter sensitive to or regulated by the Gal80p, Gal3p and Gal4p encoded by the expression constructs of steps a-c,
wherein the promoters are fused to a heterologous gene.

64. (Withdrawn) The method of claim 63, wherein Gal4p or a fragment thereof is expressed from a heterologous Gal4p gene introduced into the multicellular organisms or cells therefrom.

65. (Withdrawn) The method of claim 63, wherein the inducing agent is galactose.

66. (Withdrawn) The method of claim 63, wherein the coding sequence operably linked to a promoter sensitive to or regulated by the proteins encoded by the expression constructs of steps a-c are expressed from UAS_{GAL}-containing promoters.

67. (Withdrawn) A method of regulatable gene expression in multicellular organisms or cells therefrom comprising:

- a. introducing a recombinant expression construct encoding Gal80p into the multicellular organism or cell therefrom,
- b. introducing a recombinant expression construct encoding Gal3p into the multicellular organism or cell therefrom,
- c. introducing a recombinant expression construct encoding Gal4p or a fragment thereof fused to a transcription factor or DNA-binding portion thereof into the multicellular organism or cell therefrom,
- d. introducing a coding sequence operably linked to a promoter sensitive to or regulated by the transcription factor,
- e. treating the multicellular organism or cell therefrom with an inducing agent, wherein treatment mediates the cytoplasmic interaction of the Gal3p and Gal80p, relieving Gal80p inhibition and permitting Gal4p activation of the coding sequence operably linked to the

promoter sensitive to or regulated by the transcription factor.

68. (Withdrawn) The method of claim 67, wherein Gal4p or a fragment thereof is expressed from a heterologous Gal4p gene introduced into the multicellular organisms or cells therefrom.

69. (Withdrawn) The method of claim 67, wherein the inducing agent is galactose.

70. (Withdrawn) The method of claim 67, wherein the promoter that is sensitive to or regulated by the transcription factor is an endogenous promoter.

71. (Withdrawn) The method of claim 67, wherein the coding sequence operatively linked to the promoter that is sensitive to or regulated by the transcription factor is an endogenous coding sequence.

72. (Withdrawn) The method of claim 67, wherein the promoter that is sensitive to or regulated by the transcription factor is a heterologous promoter.

73. (Withdrawn) The method of claim 67, wherein the coding sequence operatively linked to the promoter that is sensitive to or regulated by the transcription factor is a heterologous coding sequence.

74. (Withdrawn) The method of claim 67, wherein the transcription fragment is a transcription factor from a cell of a multicellular organism.